Automutagenic Action of the Extract from Immature *Allium fistulosum* Seeds on Mitosis.

Yukio KATO

Biological Institute, Faculty of Science, Nagoya University, Nagoya

Received August 7, 1960

Spontaneous chromosome aberrations occur with high frequency in the seedling of *Allium* and *Lilium* (Kato 1951, 1954 and 1955). According to D'Amato and Hoffmann-Ostenhof (1956), the majority of chromosome aberrations which occur spontaneously is not assumed to be induced by external factors, so that their causes should be sought in the interior of cells. Some chemical substances formed naturally in the plant body may act under certain conditions as mutagens against plant itself. This is an idea of automutagenic action on mitosis. Although mutagenic metabolites are included within the cell usually in very low concentrations, it may happen under special physiological conditions that they reach such a high concentration as to give effect. The author (1954, 1957c and 1958) mentioned already the radiomimetic effects of the extracts from dry seeds of *Allium fistulosum* and *Spinacia oleracea*. In the present paper the effects of the extract from immature Welsh onion seeds are described.

Material and methods

Allium fistulosum var. "Kujōnegi" ($2n=16$), in which not mitotic abnormalities nor free fragments had been previously ascertained, was used in the present experiment.

The extract of the immature seeds was prepared by the following procedures: (1) Water extract; 2, 4 and 9 grams of immature seeds at various developmental stages were homogenized with water and shaken for 24 hours at 25°C, and then was filtered. (2) Alcohol extract; 10 grams of seeds were extracted with 50 ml. of 95 % alcohol for 24 hours, and the filtrate was evaporated to dryness on a water bath. The residue thus obtained was resuspended in 20 ml. of water. (3) Water-acetone extract; after the seeds were homogenized with water, the substances absorbed by charcoal were extracted with acetone. The residue obtained after the evaporation of acetone was suspended in 20 ml. of water. Owing to the difficulty in collecting immature seeds of the same developmental stage, the material used for extraction was a mixture of seeds at various developmental stages from watery-milk stage to nearly mature stage. The root tips were immersed in a series of aqueous suspension of the extract and the durations of treatment were 4, 24 and 48 hours. Immediately after the treatment or

"Dedicated to Prof. Hajime Matsuura and Prof. Yukio Yamada celebrating their Sexagenery birthday".

after 24–48 hours' recovery, root tips were fixed with aceto-alcohol for one hour or more. Aceto-orcein squash technique was used exclusively.

Observations

The extracts obtained by three different methods have the cytological effects, similar to each other, then the effect of water extract on mitosis is mainly described. The abnormalities observed in the present experiment is shown in Table 1.

Table 1. Cytological abnormalities observed in the root-tip cells of Welsh onion.

Duration of treatment/ Duration of recovery	4 hr.	24 hr.	48 hr.	4 hr./24 hr.	4 hr./48 hr.
Water extract 2 %	f	f	*	f	*
4 %	*	f	+	*	f
9 %	f	+	+	*	f
Alcohol extract	*	f	*	f	*
Water-acetone extract. .	*	*	+	f	*

The symbol “*” indicates the abnormalities other than radiomimetic disturbances, such as c-mitosis, contraction and stickiness of chromosomes, etc.

The symbol “f” indicates the occurrence of fragments, and “+” lethal effects suggested cytologically by clotting, clumping and pycnosis.

Of these abnormalities, three items will be described below.

1. Toxicity

In a higher concentration, the extract had a toxic effect on root meristem. The root was lost its turgor and soften by the 24–48 hours' treatment with 4 or 9 % solution. The toxicity correlated with the duration of treatment and/or with the temperature during the treatment. For instance, the 24 hours' treatment with the 9 % solution brought about the loss of root turgor at 25°C, but not at 12°C. In these lethal concentrations, clotting or clumping of chromosomes took place and the mitotic frequency was intensively depressed or completely inhibited.

2. C-mitotic reaction.

The chromosomes of the treated cell became thick and short. Such contraction was very conspicuously induced even by the treatment of a short duration.

Fig. 1 shows normal chromosomes in the control experiment, and Fig. 2 contracted chromosomes obtained by the 4 or 8 hours' treatment with the 2% solution. The degree of contraction was very variable, depending on both the concentration of the extract and the duration of the treatment. By the 8 hours' treatment with the 4 % solution, the length of chromosomes was reduced to nearly 1/3 of that of control.

The extract had no particular effects on the induction of c-mitosis nor on the destruction of the nuclear spindle. However, the typical c-mitosis was observed in the material treated for 24 hours with 4 % solution. The 8 hours' treatment induced with 4 or 9 % solution often ball-metaphase and partial c-mitosis, and in the case of complete, typical c-mitosis X- or O-shaped chromosome pairs were observed, as shown in Figs. 3 and 6. Tetraploid divisions were most frequently found in the material treated for 24 hours (Fig. 4). Spindle disturbances, such as the formation of tripolar spindle, lagging chromosomes in anaphase, disorientation of the metaphase spindle,

Figs. 1-11. The effect of extracts of immature *Allium fistulosum* seeds on the root meristem of the same species. 1. normal chromosomes in metaphase. 2, contraction of chromosomes. 9% 8 hrs. (W). 3, c-mitosis. 9 % 8 hrs. (W). 4, tetraploid metaphase. 24 hrs. (A). 5 and 6, c-mitosis and stickiness in metaphase. 24 hrs. (A). 7-11, fragmentation of chromosomes. 7, 4% 8 hrs. (W), 8, 2 % 24 hrs. (W). 9, 24 hrs. (A). 10, 24 hrs. culture after 4 hrs. treatment (A). 11, 24 hrs. culture after 4 hrs. treatment (W-A). (W)....water extract. (A)....alcohol extract. (W-A)water-acetone extract.

and wide scattering of chromosomes in metaphase (Fig. 19) were extremely rare. Restitution nuclei resulting from the difficulty of chromosome separation in anaphase were formed. Double nuclei (Fig. 22) or dump-bell shaped nuclei were frequently found in the material treated with water extracts for 24-48 hours. In addition to these phenomena, irregular-shaped or lobed nuclei as well as nuclei with tails or bridges were also found. The fused nucleus, resulting from a failure of karyokinesis, was also seen in the material treated with the 4% solution for 24 hours.

3. Chromosome fragmentation.

Generally speaking, chromosomal changes induced by the extract may be divided into two categories. The one is represented by stickiness and pseudochiasma, and the other by chromosome and chromatid breaks. The former is considered to be due to the primary effect, the latter to the secondary effect.

The stickiness of chromosomes is one of the outstanding responses. Especially

Figs. 12-23. The effect of extracts of immature *Allium fistulosum* seeds on the root meristem of the same species. 12, sticky bridges. 24 hrs. (W-A). 13, a cell with bridge and fragment. 48 hrs. culture after 9% 4hrs. treatment. 14-15, sticky bridges and fragment. 24 hrs. (A). 16 and 17, sticky bridges and fragments. 24 hrs. culture after 4 hrs. treatment (W-A). 18, severe stickiness. 9% 24 hrs. (W). 19, scattering of chromosomes in metaphase. 2 % 24 hrs. (W). 20, nuclei having bridge. 2 % 48 hrs. (W). 21, chromosomes stickiness. 24 hrs. (W-A). 22, double nucleus, 48 hrs. (A). 23, segmentation between the double nuclei. 48 hrs. (A). (W)... water extract. (A)... alcohol extract. (W-A)... water-alcohol extract.

in the 24 hours' treatment with the alcohol or water-acetone extracts, of total number of anaphasic cells observed, 20-30 % have multiple bridges.

Chromosome stickiness was also observed in metaphase, as shown in Figs. 5 and 6. These figures represent also c-pairs and partial despiralization of the chromonemata. As the sticky bridge in anaphase is not usually accompanied by a fragment, it can be distinguished from the true chromatid bridge. A breakage of the sticky bridge,

however, bring; the fragmentation of chromosomes.

Such case was frequently observed, especially in the roots treated with the alcohol extract (Figs. 14 and 15). Figs. 7–11 show various instances of single or paired acentric fragments, and Figs. 13 and 16 the cells with a fragment and bridge. Such aberrant cells were observed in the materials treated with the water-acetone extract more frequently than in those treated with other extracts.

The fragment within a cell was less than two in number, and was very various in size. The frequency of cells with the fragment was considerably low, and its correlation to the extract concentrations was not clear-cut. After the treatment of a short duration, pseudochiasma was observed as the primary effect in a low frequency, and it gave two fragments in a later stage. The micronucleus was found in some resting cells in a low frequency. Its occurrence might result from the event that the acentric fragments have failed to be included into either daughter nuclei.

Discussion

As mentioned previously, spontaneous chromosome aberrations are very frequent in the seedling of *Allium*. The water extract from dry seeds of Welsh onion has a certain radiomimetic activity. In other words, the water extract causes the breakage of chromosomes to occur, and it evidently has some mutagenic action on mitosis.

It was found from the present experiment that some chemical substances, which act on root meristem as automutagens, exist in immature seeds. Keck and Hoffmann-Ostenhof (1951) were the first in showing that the water extract from onion bulbs (*Allium cepa*) had induced the chromosomal fragmentation in root-tip cells of the same species. The substance responsible for the mutagenic effect on mitosis was identified by them as diallyl disulfide. Although the extract from *Allium sativum* bulbs was very toxic and intensively effective as mutagens against *Allium cepa* roots, it failed to show any radiomimetic effect on the root-tip cells of *Allium sativum* (Keck and Hoffmann-Ostenhof, 1955). The present author (1957c) ascertained the similar phenomenon, and came to the conclusion that the water extract of spinach fruits was cytologically less effective upon root-tip cells of spinach itself than upon those of onion. Resende (1951) has induced chromosome aberrations in root tips of *Allium cepa* by the extract from old dried scales of onion bulbs. The effective substance in this case is believed to be identical with protocatechic acid. In the present case, however, the chemical composition of the extracts was not determined.

A number of free fragments, bridges and other abnormalities (highly polyploidized mitosis, etc.) were frequently observed in the developing endosperm of *Allium* (Kato 1957a and b). The endosperm is an important part of the immature seed. Therefore, cytological abnormalities in the endosperm may be induced by aging of the tissue or by some chemical substances included within it. If the active substance is accumulated up to the critical threshold concentration under a special condition, it may induce the chromosome breakage in the endosperm. Such substance may accumulate in the primary root and radicle of the seedling. The water extract of immature *Lilium*

formolongo seeds was tested on the root tip of the same species, however, any automutagenic action on mitoses was not observed.

Summary

The extract of immature Welsh onion (*Allium fistulosum*) seeds has a radiomimetic action on mitosis of the same species. The effective substances are extractable by water, alcohol and acetone. In the developing endosperm, chromosome aberrations and high polyploidy were frequently found. It is, therefore, likely that some chemical substances responsible for radiomimetic action may accumulate within the endosperm of immature seeds. Mitotic abnormalities found in the seedling of Welsh onion are explained by assuming that some chemical substances are formed in the early stage of germination, as mentioned previously by the present author.

Acknowledgement

The author wishes to express his appreciation to Prof. T. Shimamura for his remarks during the preparation of this manuscript.

Literature cited

- D'Amato, F. and Hoffmann-Ostenhof, O. 1956. Metabolism and spontaneous mutation in plants. *Adv. Genetics*, **8** : 1-28.
- Hoffmann-Ostenhof, O. und Keck, K. 1951. Pflanzliche Stoffwechselprodukte als Mitosegifte. 11. Diallyldisulfid, ein Bestandteil der Lauchöle, als mitosestörende Substanz. *Monatsh. Chem.* **82** : 562-564.
- Kato, Y. 1951. Spontaneous chromosome aberrations in mitosis of *Allium fistulosum* L. (A preliminary note). Japanese with English summary. *Bot. Mag. (Tokyo)*, **64** : 152-156.
- 1954. Descriptive and experimental cytology in *Allium*. 11. Chromosome breakage in the seedling of *Allium*. *Bot. Mag. (Tokyo)*, **68** : 122-129.
- 1955. Polyploid mitosis, extranuclear bodies and mitotic aberrations in seedlings of *Lilium Maximowiczii* Regel. *Cytologia*, **20** : 1-20.
- 1957a. Cytological abnormalities in the embryo-sac mother cell and endosperm tissue of *Lilium formolongo* Hort. and *Allium fistulosum* L. *Cytologia*, **22** : 69-79.
- 1957b. On the endosperm nucleus of *Allium*. *Jour. Hered.* **48** : 7-10.
- 1957c. Chromosome breaks and bridges induced by extracts from spinach and beet fruits. *Phyton (Argentina)*, **8** : 131-136.
- 1958. Automutagenic action of the extracts from immature seeds on mitosis (Abstract). *Jap. Jour. Genet.* **33** : 309-310.
- Keck, K., Frisch-Niggemeyer, W., Ascher, D. und Hoffmann-Ostenhof, O. 1951. Inhaltstoffe des Knoblauchs und ihre Wirkungen. 1. Über Substanzen, welche den Austritt des Chromatins aus Zellkernen im Wurzelmeristem von *Allium*-Arten bewirken. *Monatsh. Chem.* **82** : 755-758.
- Keck, K. und Hoffmann-Ostenhof, O. 1951. Pflanzliche Stoffwechselprodukte als Mitosegifte. 1. Mitosehemmende und -störende Substanzen in wässrigen Auszügen aus *Allium cepa* (Speisewiebel). *Monatsh. Chem.* **82** : 559-562.
- und Hoffmann-Ostenhof, O. 1956. Stoffwechselprodukte von *Allium*-Arten als Mitosegifte. *Cytologia*, **21** : 208-221.
- Resends, F. 1951. Agentes modificadores do metabolismo celular. 1. Accao de neutroes, tripaflavina e extractos vegetais nas mitoses dos vertices vegetativos da raiz. *Biol. Soc. Port. Cienc. Nat.* **3** : 181-211.